AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning on page 45, line 10 as follows:

Specific antigen recognition by the variable domains underlies the pathogenic effects of certain antibodies (Abs) ~~Abs~~ produced as a result of autoimmune, allergic and anti-transplant reactions. For instance, Abs found in myasthenia gravis (reviewed in ref. 1) and hemophilia (reviewed in ref. 2) bind important epitopes of the acetylcholine receptor and Factor VIII, respectively, which interfere with the biological activity of these proteins by a steric hindrance mechanism. Other Abs utilize their Fc region to mediate pathogenic effects but antigen recognition by Ab variable domains is the stimulus initiating these effects, e.g., Ab recognition of erythrocyte antigens stimulates complement activation by the Fc region in autoimmune hemolytic anemia and incompatible blood transfusions. Similarly, allergen recognition by IgE bound to Fc receptors on the surface of mast cells stimulates their degranulation. In other diseases, the mechanism of Ab pathogenicity is less clear. For example, Abs to nucleic acids in lupus (reviewed in ref. 3) and to thyroglobulin in Hashimoto's thyroiditis (reviewed in ref. 4) are unambiguously disease-associated, but additional immune abnormalities are also evident in these diseases, and the precise functional effects of the Abs remain debatable. Recently, a novel variable domain mechanism underlying Ab pathogenicity has emerged, viz., the catalytic cleavage of antigens. Hydrolytic catalysts such as Abs to polypeptides (5-8) and nucleic acids (9) hold the potential of permanent antigen inactivation. Moreover, catalysts are endowed with turnover capability, i.e., a single Ab molecule can hydrolyze multiple antigen molecules, suggesting that such Abs may exert functional effects that are more potent than Abs dependant on stoichiometric antigen recognition. ~~.sup.1~~ Abbreviations: Ab, antibody; AMC, 7-amino-4-methylcoumarin; BSA, bovine serum albumin; CAL, covalently reactive antigen analog; DFP, diisopropyl fluorophosphate; exEGFR, extracellular domain of human epidermal growth factor receptor; KLH, keyhole limpet hemocyanin; MCA, methylcoumarinamide; V domain, variable domain; VIP, vasoactive intestinal peptide

Please amend the paragraph beginning on page 49, line 8 as follows:

Covalent Ab Labeling. Monoclonal Ab c23.5, raised by hyperimmunization with VIP. It is characterized by strong recognition of the ground state of VIP (Kd 1.9 nM; Km 0.34 nM), made possible by traditional noncovalent Ab paratope-epitope interactions (23). The catalytic site of the Ab is located in the light chain subunit and is composed of a serine protease-like catalytic triad (15). Here, we compared the covalent binding of this Ab by VIP-CAL 3 and hapten CAL 1. The isotype-matched Ab UPC10 (IgG2a, .kappa.) served as the control to determine background Ab nucleophilic reactivity independent of noncovalent recognition of VIP. The covalent reaction was visualized by boiling the reaction mixtures

followed by denaturing SDS-electrophoresis and detection of biotin-containing adducts (FIG. 15A, inset). Accumulation of covalent VIP-CAL 3 adducts with the anti-VIP Ab increased linearly as a function of time,[3] (the CAL-Ab reactions are predicted to follow the second-order rate law, and linear adduct accumulation will occur in the initial stage of the reaction) with the light chain subunit accounting for the majority of the adducts (nominal mass 29 kD determined by comparison with molecular mass standards). Adducts of VIP-CAL 3 with the control Ab were formed at lower levels. Similarly, hapten CAL 1 reacted with anti-VIP and control Abs slowly compared to the VIP-CAL, and there was no preference for covalent binding of the hapten CAL at the light chain subunit. Apparent reaction velocities (V.sub.app) were obtained from the slopes of linear regression curves fitted to the progress data by least square analysis ([Ab-CAL]=V.sub.appt, where [Ab-CAL] represents the intensity of Ab-CAL adduct band in AAU, and t, the reaction time]. V.sub.app values are compiled in Table 5. For the anti-VIP Ab, V.sub.app of the VIP-CAL 3 reaction with the light chain was 6.6-fold greater than the heavy chain. Hapten CAL 1 V.sub.app values for the two subunits of this Ab were nearly equivalent. V.sub.app for the reaction of VIP-CAL with the anti-VIP light chain was 66-fold greater than the corresponding reaction with the control Ab light chain. These observations indicate the selective nucleophilic reactivity of the ant-VIP light chain. Inclusion of VIP devoid of the phosphonate group in the reaction mixture inhibited the formation of VIP-CAL 3 adducts with the anti-VIP light chain (FIG. 15B; inhibition in 3 repeat experiments, 41.0.+-.7%). It may be concluded that selective covalent binding of VIP-CAL 3 by the anti-VIP Ab is made possible by noncovalent interactions due to the presence of the VIP sequence.[3] The CAL-Ab reactions are predicted to follow the second-order rate law, but linear adduct accumulation will occur in the initial stage of the reaction.

Please amend the paragraph on page 53, line 21 as follows:

As noted previously, catalytic Abs are proposed to contribute in the pathogenesis of autoimmune disease. Specific covalent inhibitors represent a novel means to help define the precise functional effects of the Abs. Such inhibitors may serve as prototypes for development of therapeutic agents capable of ameliorating harmful Ab effects. In addition to inactivation of secreted Abs, reagents such as the VIP-CAL may be useful in targeting antigen-specific B cells. The feasibility of this goal is indicated by evidence that CALs bind covalently to Abs expressed on the surface of B cells as components of the B cell receptor.[3] Ab nucleophilicity may be viewed as an indication of their competence in completing the first step in covalent catalysis, i.e., formation of an acyl-Ab reaction intermediate. This is supported by observations that the magnitude of Ab nucleophilic reactivity is correlated with their proteolytic activity (31). A recent study suggests that noncatalytic Abs also contain nucleophiles but are

unable to facilitate steps in the catalytic cycle following covalent attack on the antigen, viz., water attack on the acyl-Ab intermediate and product release (31). Regardless of the physiological functions of nucleophiles expressed by noncatalytic Abs, their presence may allow CAL-targeting of Ab populations with established pathogenic roles, e.g., anti-factor VIII Abs in hemophilia.[2]

Please add the following paragraph to the specification after page 89, line 28:

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